



Autologous stem-cell transplantation in treatment-refractory Crohn's disease: an analysis of pooled data from the ASTIC trial

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Abstract: **BACKGROUND** The randomised controlled ASTIC trial showed no benefit of mobilisation and autologous haematopoietic stem-cell transplantation (HSCT) compared with mobilisation followed by conventional therapy using a stringent primary endpoint (steroid-free clinical remission for 3 months with no endoscopic or radiological evidence of intestinal inflammation) in patients with treatment-refractory Crohn's disease. We now assess HSCT in patients enrolled in the ASTIC trial using endpoints that are traditional for clinical trials in Crohn's disease, and identify factors that predict benefit or harm. **METHODS** Patients who underwent mobilisation and were randomly assigned to conventional therapy in the ASTIC trial were offered HSCT at 1 year and underwent complete assessment for a further year. We report analyses of the combined cohort of patients who underwent HSCT at any time during the ASTIC trial programme. The primary outcome for this analysis was 3-month steroid-free clinical remission at 1 year after HSCT (Crohn's Disease Activity Index [CDAI] <150). We also examined the degree of endoscopic healing at 1 year. Multivariate analysis was performed to identify factors associated with achieving the primary endpoint by using logistic regression, and factors associated with experiencing a serious adverse event using Poisson regression. Participants were not masked to treatment, but the adjudication panel that reviewed radiology and endoscopy was masked to allocation and visits. All patients who underwent HSCT and had data available at baseline and 1-year follow-up were included in the primary and safety analysis. This trial is registered with ClinicalTrials.gov, number NCT00297193. **FINDINGS** Between June 28, 2007, and Sept 1, 2011, 45 patients were enrolled in the ASTIC trial from 11 European transplant units. 23 patients were randomly assigned to immediate HSCT, and 22 patients were assigned to mobilisation followed by conventional care. After completion of the ASTIC trial, 17 patients from the conventional care group received HSCT. In the combined cohort, data were available for 40 patients at baseline and 38 patients at 1 year after HSCT (one patient died, one withdrew). At 1 year after HSCT, 3-month steroid-free clinical remission was seen in 13 (38%, 95% CI 22-55) of 34 patients with available data for the whole year. Complete endoscopic healing was noted in 19 (50%, 34-66) of 38 patients. On multivariate analyses, factors associated with the primary outcome were short disease duration (odds ratio [OR] 0·64, 95% CI 0·41-0·997 per year; $p=0\cdot048$) and low baseline CDAI ($0\cdot82$, $0\cdot74$ - $0\cdot98$ per 10 units; $p=0\cdot031$). 76 serious adverse events occurred in 23 of 40 patients with available data. The most common serious adverse event was infection, most of which were treatment related. Smoking and perianal disease at baseline were independent factors associated with the number of serious adverse events (OR 3·07 [95% CI 1·75-5·38; $p=0\cdot0001$] for smoking and 3·97 [2·17-7·25; $p<0\cdot0001$] for perianal disease) on multivariate analysis. **INTERPRETATION** When assessed using endpoints traditional for clinical trials of conventional therapy in Crohn's disease, HSCT resulted in clinical and endoscopic benefit, although it was associated with a high burden of adverse events. The prognostic factors identified could allow the therapy to be targeted to patients most likely to benefit and not experience serious adverse events. **FUNDING** Broad Medical Research Program, National Institute for Health Research Senior Investigator Award, The University of Nottingham Medical School Dean's Fund, and The Nottingham University Hospitals NHS Trust Research and Development Fund.

DOI: [https://doi.org/10.1016/S2468-1253\(17\)30056-0](https://doi.org/10.1016/S2468-1253(17)30056-0)

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-145566>

Journal Article

Accepted Version



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Originally published at:

Lindsay, James O; Allez, Mathieu; Clark, Miranda; Labopin, Myriam; Ricart, Elenor; Rogler, Gerhard; Rovira, Montserrat; Satsangi, Jack; Farge, Dominique; Hawkey, Christopher J; ASTIC trial group; European Society for Blood and Marrow Transplantation Autoimmune; European Crohn's and Colitis Organisation (2017). Autologous stem-cell transplantation in treatment-refractory Crohn's disease: an analysis of pooled data from the ASTIC trial. *Lancet Gastroenterology Hepatology*, 2(6):399-406.

DOI: [https://doi.org/10.1016/S2468-1253\(17\)30056-0](https://doi.org/10.1016/S2468-1253(17)30056-0)

One year outcome of autologous stem cell transplantation in treatment refractory Crohn's disease: identifying factors that predict benefit and harm

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Key Words: Crohn's disease, haematopoietic stem cell transplantation, anti TNF therapy

Word count:

ABSTRACT

BACKGROUND: To assess the outcome of autologous haematopoietic stem cell transplantation (HSCT) in refractory Crohn's disease and identify factors that predict benefit or harm.

METHODS: All patients undergoing HSCT either during or after the controlled ASTIC trial were included. Clinical, radiological and endoscopic outcomes were assessed at baseline and one year. The primary outcome for this analysis was 3 months steroid free clinical remission at one year. Complete endoscopic healing was defined as an SES-CD ulceration subscore of 0 in all segments. An adjudication panel blinded to allocation and visit reviewed radiology and endoscopy.

FINDINGS: Evaluable data was available for 40 patients at baseline and 38 patients at one-year (one patient died, one withdrew). At one year, 3 months steroid free clinical remission was seen in 38.2% (n=13/34) and complete endoscopic healing in 50% (19/38). Compared to baseline, there were significant improvements at 1 year for CDAI, PRO2, quality of life and SES-CD. After multivariate analyses factors associated with the primary outcome were short disease duration (OR: 0.64; 95% CI: 0.41-0.997 per year; p=0.048), low baseline CDAI (OR: 0.82; 95% CI: 0.74-0.98 per 10 units; p=0.031) and a trend for higher baseline SES-CD (OR: 0.85; 9% CI: 0.71-1.002); p=0.053 per unit). 76 serious adverse events occurred in 23 patients. Smoking and perianal disease at baseline were independent factors associated with the number of SAEs (OR (95% CI) of 3.07 (1.75-5.38; p=0.0001) and 3.97 (2.17-7.25; p=0.00001).

INTERPRETATION: HSCT results in clinical and endoscopic benefit, despite a high burden of adverse events in refractory Crohn's disease patients. Identification of prognostic factors will allow the therapy to be targeted.

FUNDING: Broad Medical Research Program, National Institute for Health Research Senior Investigator Award, The University of Nottingham Medical School Dean's Fund and The Nottingham University Hospitals NHS Trust Research & Development Fund.

RESEARCH IN CONTEXT

Evidence before this study

Chronic active Crohn's disease refractory to therapy is associated with significant morbidity and major impact on patient quality of life and work productivity. Identified case reports (Pubmed English language search of [Stem cell transplant and Haematopoietic] and [Colitis or Crohn* or IBD]) suggest clinical and endoscopic improvement with HSCT in this patient group. One randomised controlled trial (ASTIC trial) reported no benefit of HSCT over mobilisation alone at one year for the ambitious primary endpoint of clinical remission off therapy for 3 months with no endoscopic or radiological evidence of intestinal inflammation. There were a high number of serious adverse events in patients undergoing HSCT as well as in patients continuing on conventional care.

Added value of this study

We assess clinically relevant outcomes from all CD patients undergoing HSCT as part of the ASTIC trial program (either during the RCT or after the primary endpoint), which comprise the largest cohort in the literature. HSCT is associated with significant improvement in clinical disease activity, quality of life and endoscopic disease activity after one year. A short disease duration and endoscopic evidence of active disease are associated with clinical remission for 3 months off steroids at one year. Smoking and presence of perianal disease are factors associated with serious adverse events.

Implications of all the available evidence

This additional analysis of all patients undergoing HSCT during the ASTIC trial program demonstrates that HSCT has a significant impact on clinical and endoscopic disease activity and identifies factors that predict response as well as harm. Further clinical trials of HSCT in selected patients using more traditional primary endpoints are justified. These should include low intensity mobilisation and conditioning regimens to assess whether a more favorable safety profile can be achieved whilst maintaining clinical efficacy.

INTRODUCTION

Treatment refractory Crohn's disease (CD) results in chronic ill health, reduced life expectancy and often requires surgery.¹⁻³ Progressive inflammation and surgery result in digestive tract damage that manifests with strictures, short bowel syndrome and stoma formation and is associated with refractory non-inflammatory gastrointestinal symptoms.^{1,4} Although appropriate use of conventional and biologic therapies can induce mucosal healing, a significant number of patients experience primary or secondary non-response.^{1,5} Patients with treatment refractory CD are prepared to undergo a significant treatment related risk to achieve remission.⁶ There has been considerable interest in case reports that autologous haematopoietic stem cell transplantation (HSCT) can induce sustained disease remission in such patients.⁷⁻¹³

The ASTIC trial compared outcome at one year in 23 patients with refractory CD after autologous HSCT to 22 undergoing stem cell mobilisation alone.¹⁴ This was a negative trial and few patients in either arm achieved the ambitious primary endpoint of clinical remission off therapy for 3 months with no endoscopic or radiological evidence of intestinal inflammation. However, exploratory analyses revealed that more HSCT patients came off immunosuppressive therapy, achieved clinical remission and were free of endoscopic disease than those who had mobilisation alone. HSCT was associated with a significant number of serious adverse events (SAEs) and one patient died. However, there was also a significant morbidity associated with conventional therapy.

It is crucial to balance the benefits and risks of HSCT against on-going partially effective conventional therapy. The identification of factors that predict short-term benefit or harm from HSCT will inform future clinical trials and allow therapy to be targeted. Patients who underwent mobilisation and were then randomised to conventional therapy in ASTIC were offered HSCT at one year and underwent complete assessment for a further year. This manuscript reports the clinical and safety analysis of the combined cohort of patients who underwent HSCT at any time during ASTIC and determines factors associated with clinically relevant outcomes.

METHODS

Study Design

ASTIC was a parallel group RCT designed to evaluate the benefit and safety of autologous stem cell mobilisation and HSCT compared to mobilisation followed by conventional therapy at one year in patients with refractory Crohn's disease.¹⁴ Few patients in either group met the stringent primary endpoint of clinical remission off all medication for 3 months with no evidence of active ulceration on endoscopic or radiological assessment of the intestine. Patients randomised to conventional therapy after mobilisation were offered HSCT after [primary outcome assessment and end of follow-up](#). The trial was conducted in six European countries at eleven centres approved for allogeneic transplantation by JACIE (Joint Accreditation Committee of the International Society for Cellular Therapy) and the European Society for Blood and Marrow Transplantation (EBMT).^{15,16} This manuscript reports the clinical, radiological and endoscopic outcomes from all patients with evaluable data at one year after HSCT. It includes patients initially randomised to an early transplant and those who subsequently underwent HSCT after conventional therapy.

Participants

The inclusion / exclusion criteria and recruitment process for the ASTIC trial have been reported.¹⁴ Briefly, participants were aged 18 to 50 years with continuing treatment refractory CD not amenable to surgery and impaired quality of life (Inflammatory Bowel Disease Questionnaire (IBDQ),¹⁷ or European Quality of Life Visual analogue scale (EQ-VAS))¹⁸ despite at least three immunosuppressive/biological agents and corticosteroids. The first patient was consented on June 28th 2007 and the last on September 1st 2011, and final date of follow-up included in the current analysis was 4th February 2014.

Ethical Issues

A multidisciplinary trial steering group (TSG) accepted patients for inclusion and all patients provided written informed consent. The protocol was approved by the institutional review board at each site and complied with country-specific regulatory requirements. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.¹⁹ An independent data and safety monitoring committee (DSMC) reviewed safety data after every ten patients randomised or in the event of death or other concerns.

Mobilisation and conditioning regimen

Consented patients underwent protocol designated screening investigations that were reviewed by the TSG. Eligible patients underwent stem cell mobilisation using cyclophosphamide $2\text{gm}/\text{m}^2$ x two days and non-glycosylated Granulocyte Colony Stimulating Factor (G-CSF, filgrastim) $10\text{ }\mu\text{g}/\text{kg}/\text{day}$.¹⁴ Patients underwent leukapheresis when the CD34+ count exceeded $20 \times 10^4/\text{ml}$ to a target of $3\text{--}8 \times 10^6$ CD34+ cells/kg body weight and if successful underwent HSCT immediately or after a delay of one year. An intermediate intensity conditioning regimen was used which comprised intravenous cyclophosphamide $50\text{ mg}/\text{kg}/\text{day}$ x four days and, from day three, rabbit antithymocyte globulin (Genzyme) $2.5\text{ mg}/\text{kg}/\text{day}$ and methylprednisolone $1\text{ mg}/\text{kg}/\text{day}$ x three days, with infusion of unselected autologous stem cells (minimum 3×10^6 CD34+ cells/kg) on day seven.¹⁵ All patients were followed for one year after HSCT and could receive standard care for CD during this period, including corticosteroids, immunosuppressive agents and biological therapy that were subsequently withdrawn to protocol if appropriate.

Assessments and time points

All patients underwent the same schedule of assessments prior to and over the year following HSCT. This comprised (i) disease activity, haematology, biochemistry, adverse events according to EBMT guidelines,¹⁵ concomitant medication every 6 weeks (ii) quality of life (IBD-Q and EQ-5D) every 6 months and (iii) endoscopic and radiological assessment of the entire intestine at baseline and one year. Baseline data was recorded prior to mobilisation in the patients undergoing immediate HSCT and prior to conditioning in those undergoing HSCT at one year. Outcome data were recorded one year after conditioning for all patients. An adjudication committee who were blinded to time of assessment and prior treatment assignment reviewed all radiology and endoscopy reports to determine intestinal disease activity.

Endpoints and outcome

Steroid free clinical remission was defined as a Crohn's disease Activity Index (CDAI) <150 with no corticosteroids for at least 3 months.²⁰ A 2-point patient reported outcome (PRO2) was recorded.²¹ Complete endoscopic healing was defined as an ulceration sub score of 0 in all segments using the Crohn's disease simple endoscopic score (SES-CD).²² Partial endoscopic healing was defined as an ulceration sub score of no more than 1 in ≤ 2 segments of all segments examined. Patients were defined free from active disease if there was no ulceration on upper GI endoscopy, colonoscopy and small bowel imaging assessed by the adjudication committee. Endoscopic disease regression was defined as an SES-CD score of 0 in all segments.

Statistical analysis

The primary endpoint was steroid free clinical remission. Secondary endpoints included clinical and endoscopic disease activity) and quality of life. Quantitative variables were described using mean and standard error (SE) and compared in univariate analysis using paired or non-paired t-test. Categorical variables were described with counts and percent and compared by Chi-square test or Fisher's exact test. Multivariate analyses were performed using logistic regression for all endpoints apart from the number of SAEs, which was analysed using Poisson regression. All associated factors in univariate analyses with a p-value less than 0.10 and factors known as influencing the outcome were entered in the model. A stepwise selection of the variables was applied to develop a score for the primary endpoint according to the number of risk factors present. The optimal threshold defining high versus low values for each continuous variable in the final model was obtained using the Hothorn and Zeileis method. All tests were two-sided and p-values ≤ 0.05 were considered as indicating significant association. Analyses were performed using SPSS 22.0 and R 3.2.3 (R Development Core Team, Vienna, Austria) software packages.

RESULTS

Patients

Evaluable data is available for 40 patients at baseline and 38 patients at one-year. One patient died 20 days after conditioning and one withdrew at 26 weeks (supplementary Figure 1). The mean (SE) disease duration was 13.95 (0.94) years (Table 1). All but one patient (oesophageal disease only) had colonic or ileo-colonic involvement; 20 patients also had disease proximal to the ileum. At baseline, patients had markedly active treatment with a mean (SE) CDAI of 332.6 (17.61) and PRO2 of 23.85 (1.64) (Table 1). There was endoscopic evidence of active disease with a mean (SE) SES-CD of 14.03 (1.42).

Impact of HSCT on clinical and endoscopic disease activity

HSCT resulted in a significant improvement in clinical disease activity with a reduction in mean (SE) CDAI from baseline to one year (336.7 (18.5) to 196 (21.9); $p < 10^{-4}$). This improvement was evident at week 6 (CDAI 212.3 (22.7); $p < 10^{-4}$ compared to baseline) at which time 38% (95% CI 21%-54%) were in clinical remission (CDAI < 150) (Figure 1). HSCT improved disease specific quality of life with a significant increase in mean (SE) IBDQ from baseline to one year (119.66 (6.12) to 152.23 (8.24); $p < 10^{-4}$). There were similar significant improvements in EQ5D scores (Table 2).

There was a significant improvement in endoscopic disease activity with a reduction in mean (SE) total SES-CD from baseline to one year (14.1 (1.5) to 5.4 (1.1); $p < 10^{-4}$). Complete endoscopic disease regression was seen in 10 patients. The impact of HSCT on individual ileal and colonic segments is shown in Figure 2. Disease in the small bowel proximal to the ileum was present in 7 patients at baseline, of which 5 were adjudicated to be free from disease in this location at one year. HSCT had no impact on perianal disease.

In the year following HSCT, reintroduction of anti-TNF therapy was required in seven (18%) patients after a median (range) of 18 (14-39) weeks. This resulted in a significant reduction in mean (SE) CDAI (319 (55) to 174 (39); $p = 0.016$) and five (71.4%) patients experienced a clinical response (reduction of CDAI > 70 points).

Impact of HSCT on clinically relevant outcomes

The primary outcome of steroid free clinical remission for three months occurred in 38.2% ($n = 13/34$). In addition, 35.3% ($n = 12/34$) were in remission for 3 months off all medical therapies and 43.2%

patients (n=16/37) were in steroid free remission at one year. Complete and partial endoscopic healing was achieved in 50% (19/38) and 81.6% (n=31/38) patients respectively (Figure 3). Finally 47.4% (n=18/38) patients were adjudicated to be free from evidence of intestinal ulceration on endoscopic and radiological assessment at one year.

Baseline factors that predicted clinical and endoscopic remission at one year

Univariate analysis identified baseline factors associated with achieving the primary outcome at one year. Categorical variables (Table 3a) significantly associated with higher rate of 3 months steroid free clinical remission were disease localisation (colonic disease) and disease behaviour at baseline (inflammatory phenotype) whilst continuous variables (Table 3b) included shorter time from diagnosis to HSCT, lower baseline CDAI and higher baseline SES-CD.

After multivariate analyses, factors associated with steroid free remission for at least 3 months were shorter time from diagnosis to HSCT (OR: 0.64; 95% CI: 0.41-0.997 per year; p=0.048), lower baseline CDAI (OR: 0.82; 95% CI: 0.74-0.98 per 10 units; p=0.031) and a trend for higher baseline SES-CD (OR: 0.85; 9% CI: 0.71-1.002); p=0.053 per unit). The optimal thresholds were 298.3 for CDAI, 17 for SES-CD and 11.24 months for time from diagnosis to HSCT. This allowed a prognostic score to be devised based upon the number of predictive factors present at baseline (Table 4) leading to a ROC AUC equal to 0.948.

Univariate analysis identified low haemoglobin (mean (SE) 12.0 (0.31) vs 12.9 (0.27) g/dl; p=0.029), high CRP (31.95 (7.71) vs 15.49 (3.31) mg/l; p=0.063) and a short time from diagnosis to HSCT (12.1 (0.9) vs 16.3 (1.6) years; p=0.026) as baseline factors positively associated with complete endoscopic healing. However, no factors remained significant in multivariate analysis. Likewise, by univariate analysis, low haemoglobin (12.0 (0.32) vs 12.9 (0.26) g/dl p=0.038), high CRP (31.95 (8.21) vs 16.42 (3.25) mg/l; p=0.094) and a short time from diagnosis to HSCT (11.88 (0.93) vs 16.27 (1.52) years; p=0.021) were baseline factors associated with being adjudicated free of all active disease at one year. However, no factors remained significant in multivariate analysis.

Serious adverse events

Serious adverse events (SAEs) were common amongst patients undergoing HSCT (23 patients experienced 76 SAEs): Table 5. Univariate analysis did not reveal any baseline factors significantly associated with experiencing at least one SAE, although there was a trend that patients with perianal disease were more likely to experience a SAE (81.8% compared to 48.3%; p=0.079). In contrast,

univariate analysis of baseline factors associated with the number of SAEs experienced by patients revealed an increased mean (SE) number in current smokers (4 (1.4) vs 1.38 (0.37); $p=0.011$), patients with perianal disease at baseline (4.09 (1.19) vs (1.07 (0.28); $p=0.0004$) and patients who received conditioning and HSCT immediately after mobilisation (2.61 (0.69) vs 0.94 (0.29); $p=0.01$). Multivariate analysis identified that smoking and perianal disease at baseline were independent factors associated with the number of SAEs with OR (95% CI) of 3.07 (1.75-5.38; $p=0.0001$) and 3.97 (2.17-7.25; $p=0.00001$) respectively.

DISCUSSION

This manuscript describes the clinical and endoscopic outcomes one year after HSCT in the largest reported cohort of patients with treatment refractory CD to date. It demonstrates highly significant improvements in clinical disease activity (CDAI), QOL and endoscopic disease activity (SES-CD) between baseline and one year. Importantly, this study identifies baseline factors that predict which patients are more likely to achieve prolonged steroid free remission and also suffer serious adverse events.

ASTIC was a controlled trial with all patients undergoing mobilization before randomization to either conditioning with stem cell rescue or conventional care. Few patients in either group achieved the ambitious primary endpoint; there was a high burden of SAE and one patient died from sinusoidal obstructive disease after conditioning. As such it has been reported as a negative trial.¹⁴ Several important issues have been raised that impact on interpretation of the benefit of HSCT in the ASTIC trial.^{23,24} The predefined primary endpoint (cure) was the most stringent ever used for a clinical trial in CD. The low frequency of patients attaining it demonstrates that cure is not a common outcome and suggests the trial was underpowered. However, a significant benefit of HSCT versus mobilisation alone was noted for individual components of the primary outcome. Finally, the ASTIC trial recruited a very treatment refractory cohort and included patients with heterogeneous disease distribution, 6 (15%) of whom had diverting or permanent ileostomies.

In ASTIC, patients randomised to conventional care underwent conditioning and subsequent HSCT at the end of one year. An identical schedule of clinical, endoscopic and radiological assessments was performed over the subsequent year. Therefore, assessment of baseline and one-year outcome from the combined group of patients in the ASTIC trial irrespective of timing of HSCT has allowed a robust assessment of the impact of stem cell transplantation. Importantly it includes sufficient numbers to identify factors that predict clinical benefit. The potential for bias inherent in an 'open label' assessment is reduced by robust nature of the data collection and monitoring that was required for a controlled trial. In addition, all endoscopic and radiological investigations were reviewed and scored by an adjudication panel who were blinded to the timing of the assessment and the treatment assignment of the patient. The present analysis demonstrates that HSCT is associated with an early and sustained reduction in clinical disease activity and allowed withdrawal of steroid therapy. Patient reported outcomes and assessment of both disease specific and generic quality of life mirror the improvements in disease activity. There was a significant reduction in endoscopic disease activity and complete endoscopic healing was achieved in half of patients at one year. Complete healing

throughout the entire intestine was observed in 48.4% of patients. These figures compare favourably with reports of currently licenced and emerging biological therapies.^{25–28}

Univariate analyses highlighted baseline factors associated with achieving clinical remission off steroids for greater than 3 months. These included colonic disease, short disease duration, evidence of inflammatory rather than complex disease as well as a high endoscopic burden of disease activity. Interestingly a high baseline CDAI was a negative predictive factor, this may reflect the fact that patients with advanced disease have structural damage to the intestine that drives non-inflammatory symptoms. This concept is supported by the fact that several patients with no change to their CDAI during the trial were adjudicated to have complete regression of intestinal ulceration on radiology and endoscopy at one year. Multivariate analysis confirmed the independence of disease duration, endoscopic evidence of disease activity and a lower baseline CDAI. This allowed a predictive model to be constructed. Validation of these factors in a second large cohort with identification of the optimal cut off values for predicting response is required.

All patients were intolerant or refractory to at least one anti TNF prior to entering the trial. Patients who experienced a disease flare after HSCT were able to recommence anti TNF therapy. This resulted in a significant reduction in disease activity with over 70% experiencing short term clinical remission. Further study is required to determine the mechanism by which prior loss of response or intolerance to therapy is overcome and assess the benefit of routine maintenance therapy after HSCT.

Many patients experienced SAEs throughout each phase of the trial highlighting the importance of careful patient selection. There was a reduction in the incidence of SAEs during the course of the trial (data not shown) implying that centres became more proficient at managing HSCT with experience.²⁹ It is relevant to note that patients in both groups received 4g/m² cyclophosphamide at mobilisation. Although this was accepted practice at the initiation of the trial,¹⁵ lower intensity mobilisation regimes are currently recommended to reduce the toxicity of the procedure.^{10,23} Importantly, patients who proceeded immediately to conditioning and stem cell rescue experienced more SAEs on univariate analysis that is likely due to the additional doses of cyclophosphamide received within a short time frame. Smoking and perianal disease were independent risk factors for experiencing multiple SAEs. Studies of HSCT in other diseases have also reported that smoking is a risk factor for adverse events.³⁰ Further work is required to determine whether lower intensity mobilisation and conditioning regimes deliver similar benefits with reduced risks.

The data from this cohort represents the largest report of patients with refractory CD undergoing autologous HSCT. It demonstrates significant benefit from HSCT in terms of steroid free clinical remission, enhanced QOL and mucosal healing. The high magnitude of SAE experienced by patients suggests that this treatment strategy should only be considered in patients refractory to biologic therapies. The identification of factors that predict both benefit and harm will be invaluable in the design of future trials. Such trials should assess whether the use of low intensity mobilisation and conditioning regimes can maintain the observed benefit whilst reducing the risk of HSCT.

ACKNOWLEDGEMENTS

Financial and Material Support:

The Broad Medical Research Program

Professor Hawkey's National Institute for Health Research Senior Investigator Award

The University of Nottingham Medical School Dean's Fund

The Nottingham University Hospitals NHS Trust Research & Development Fund

Sponsors: European Group for Blood and Marrow Transplantation (EBMT) Autoimmune Diseases Working Party and the European Crohn's and Colitis Organisation (ECCO)

Role of the sponsors: The Broad Foundation reviewed and evaluated the original grant application. Listed co-authors who were members of EBMT and ECCO contributed to the design and conduct of the study, collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication

AUTHOR CONTRIBUTIONS

Dr Lindsay had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. He prepared the initial draft of the manuscript.

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Obtained funding: Hawkey CJ, Farge D

CONFLICT OF INTEREST DISCLOSURES

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Hawkey reported receiving a National Institute for Health Research Senior Investigator Award and receiving funding from the University of Nottingham Medical School Dean's Fund and the Nottingham University Hospitals NHS Trust Research and Development Fund. No other authors reported disclosures.

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FIGURE LEGENDS

Figure 1: Impact of HSCT on clinical disease activity

(A) The absolute Crohn's disease activity index (CDAI) in patients throughout the one year after HSCT.
(B) The percentage of patients in clinical remission as defined by CDAI <150 at each clinic visit during the year after HSCT

Figure 2: Assessment of endoscopic healing (SES-CD).

For each patient, ileocolonoscopy at baseline and one year after HSCT is shown. The colour in each segment indicates absence of ulceration (green), aphthous ulcer < 5 mm (yellow), large ulcers (5 mm to 20 mm) (red), very large ulcers (>20 mm) (dark red). Unexplored segments are indicated in blue, and previously resected segments in grey. Six patients had a stoma: the location of stoma is indicated.

Figure 3: Clinically relevant endpoints

The percentage of patients achieving clinical remission (CDAI<150), clinical remission over the last 3 months, normal quality of life (IBDQ>170), complete endoscopic healing (SES-CD ulcer sub-score of 0 in all segments), partial endoscopic healing (SES-CD ulcer sub-score of no more than 1 in ≤2 segments) and adjudicated free of disease (no evidence of ulceration on OGD, small bowel imaging and ileocolonoscopy) at one year after HSCT.

Supplementary Figure 1: Consort diagram

In the original ASTIC trial 132 patients were screened for eligibility, 99 were provisionally approved by the TSC, 62 signed consent forms and 50 proceeded to be registered for trial inclusion having met all criteria for inclusion. Forty-eight underwent mobilisation, and 45 proceeded to randomisation. Twenty-three patients underwent early HSCT and 22 continued conventional therapies. One of the delayed HSCT patients withdrew consent immediately after randomisation, 3 underwent surgery, one improved such that no intervention was required and 17 underwent HSCT (6 at an accelerated time point due to disease deterioration and 11 at one year as planned). Data one-year post HSCT is available for 21 of the early HSCT patients (one died 20 days after the start of conditioning and one withdrew from follow up at 26 weeks) and all 17 of the delayed HSCT patients. Therefore, evaluable data is available from 40 patients prior to HSCT (baseline) and 38 patients one year after HSCT.

Table 1: Demographic features and disease characteristics at baseline

	N=40
Age at baseline, years (mean, SE)	33.75 (1.35)
Female N (%)	23 (57.5%)
Body mass index ^a (mean, SE)	23.58 (0.74)
Current smoker N (%)	8 (20%)
Ex smoker N (%)	13 (32.5%)
Family history ^b N (%)	5 (12.5%)
Number of previous operations for CD (median, IQR)	2 (0.75-3.25)
Ileostomy N (%)	6 (15%)
Age at diagnosis, , (mean, SE)	19.9 (1.19)
Disease duration, (mean, SE)	13.95 (0.94)
Extra-intestinal involvement	
Joints N (%)	23 (57.5%)
Skin N (%)	5 (12.5%)
Eyes N (%)	7 (17.5%)
Prior Drugs	
Azathioprine / 6-Mercaptopurine N (%)	39 (97.5%)
Methotrexate N (%)	33 (82.5%)
Anti-TNF agents ^c N (%)	40 (100%)
Other immune suppressants ^d N (%)	15 (37.5%)
Number (median, IQR)	5 (4-5)
Months used (median, IQR) ^e	123 (55.1-199.3)
Disease activity	
CDAI ^f (mean, SE)	332.6 (17.61)
PRO2 ^g (mean, SE)	23.85 (1.64)
Laboratory results^h	
Haemoglobin g/dL (mean, SE)	12.5 (0.21)
Platelets x 10 ⁹ /L (mean, SE)	322.6 (20.43)
Albumin g/dL (mean, SE)	36.2 (0.87)
CRP mg/L (mean, SE)	22.5 (3.86)
Quality of Life and functional status	
EQ-VAS (0 – 100) ⁱ (mean, SE)	53.97 (3.33)
IBDQ (32 – 224) ^j (mean, SE)	120.19 (5.08)
Ileocolonoscopy evaluation	
Segments examined (max 5) (median, IQR) ^k	3.5 (2-5)
SES-CD score ^l (mean, SE)	14.03 (1.42)

- Body mass index: Weight in kilograms divided by square of the height in metres
- Family history of inflammatory bowel disease (any degree) based on patient report.
- Most patients had used more than one anti-TNF agent, including certolizumab (used in 8 patients)
- Fifteen patients received 18 other immunosuppressive/anti-inflammatory drugs (cyclosporine 4; mycophenolate 4; tacrolimus 4; thalidomide 4; vedolizumab 1; natalizumab 1).
- Summation of durations of use for each drug (some of which may have been concurrent). Where data were imprecise (e.g. year of starting or finishing only) we averaged the maximum and minimum possible duration
- CDAI: Crohn disease Activity index: an 8 component index based on number of liquid or soft stools, abdominal pain, general well-being, complications, use of anti-diarrhoeal medication, abdominal mass,

haematocrit <0.47 (men) or 0.42 (women) and % deviation from standard weight. Remission up to 150; higher values (typically up to 600) indicate active disease[Best]

- g. PRO2: a patient reported outcome measure that comprises the stool frequency and pain score from the CDAI [21]
- h. Normal ranges vary slightly between centres. Nottingham normal values are: Haemoglobin 13.0-18.0 g/dL (men), 11.5-16.5 g/dL (women); Platelets 150-450 x 10⁹ / L; Albumin 30.0-45.0 g / dL; C-reactive protein (CRP) 0-5 mg / L.
- i. EQ-VAS: European Quality of Life visual analogue scale. Self-rated health status using a visual analogue scale, which records the subject's perceptions of their own current overall health and can be used to monitor changes with time. Values range from 0-100. Higher scores indicate better quality of life (impaired quality <85)[18]
- j. IBDQ: Tool to measure health-related quality of life in adult patients with inflammatory bowel disease, with 32 questions scored in four domains: bowel symptoms, emotional health, systemic systems, social function. Values range from 32-224 Higher scores indicate better quality of life (impaired quality of life is defined as an IBDQ<170)[17]
- k. The Simple Endoscopic Score for Crohn's disease (SES-CD) summation of scores for involvement, ulceration, ulcer size and stricturing each on a 3 point scale in each of ileum, ascending colon, transverse colon, left colon and rectum. Theoretical maximum score 60 (higher is worse) [22]

Table 2: Baseline and one year scores for clinical, quality of life and endoscopic endpoints using a paired analysis including data only when available for a patient at both baseline and one year

Table 2 A	N	Baseline	One year	p value (paired)
CDAI	37	336.73 (18.46)	195.95 (21.91)	<10 ⁻⁴
PRO2	37	24.03 (1.74)	12.45 (1.61)	<10 ⁻⁴
EQ5D index	27	0.752 (0.099)	0.801 (0.035)	0.033
EQVASC	29	53.55 (3.98)	72.72 (4.18)	0.00016
IBDQ	30	119.57 (6.12)	152.23 (8.24)	<10 ⁻⁴
SESCD	36	14.11 (1.5)	5.44 (1.1)	<10 ⁻⁴

Table 3a: univariate analysis of categorical variables associated with being in corticosteroid free clinical remission for at least 3 months.

		CDAI<150 for 3 months off steroids		p (chi2 or Fisher)
		No	Yes	
Localisation	Colonic	1 16.7%	5 83.3%	0.024
	Ileal	3 100.0%	0 0.0%	
	Ileocolonic	17 68.0%	8 32.0%	
Stage at baseline (BL)	Inflammatory	7 38.9%	11 61.1%	0.014
	Stricturing	8 88.9%	1 11.1%	
	Penetrating	6 85.7%	1 14.3%	
Rx	A	12 63.2%	7 36.8%	0.85
	B	9 60.0%	6 40.0%	
Perianal disease BL	No	13 54.2%	11 45.8%	0.25*
	Yes	8 80.0%	2 20.0%	
Stoma BL	No	18 62.1%	11 37.9%	1*
	Yes	3 60.0%	2 40.0%	
Current smoker	No	18 62.1%	11 37.9%	1*
	Yes	3 60.0%	2 40.0%	
NOD2 WT	No	5 45.5%	6 54.5%	0.21
	Yes	15 68.2%	7 31.8%	

Table 3 b: Univariate analysis of continuous variables associated with being in corticosteroid free clinical remission for at least 3 months.

Mean (SE) N	CDAI<150 off steroids for 3 months		p (t-test)
	No	Yes	
Age at BL (years) N	35.74 (1.81) 21	30.67 (2.27) 13	0.091
BMI at baseline (kg/m ²) N	19.86 (1.8) 21	19.85 (2.78) 13	0.996
CRP at baseline (mg/l) N	26.57 (6.6) 19	21.54 (6.49) 12	0.612
Haemoglobin at baseline (g/dl) N	12.21 (0.26) 21	12.61 (0.41) 13	0.401
Albumin at baseline (g/dl) N	33.92 (2.04) 12	37.52 (1.82) 7	0.251
Time from mobilisation to HSCT (months) N	7.05 (1.52) 21	5.01 (1.40) 13	0.332
Time from diagnosis to HSCT (years) N	15.56 (1.06) 21	11.1 (1.79) 13	0.029
Nr. of previous resections N	2.76 (0.41) 21	1.62 (0.69) 13	0.137
CDAI at baseline N	378.65 (22.08) 21	232.98 (37.54) 13	0.001
SES CD at baseline N	10.76 (1.37) 21	21.73 (2.98) 11	0.001

Table 4: Categorisation of the 3 prognostic factors (short disease duration, low baseline CDAI and high SESCD) according to the mean

		CDAI<150 off steroids for 3 months	
		No	Yes
Number of risk factors	0 - 1	0 (0%)	8 (100%)
	2	8 (72.7%)	3 (27.3%)
	3	13 (100%)	0 (0%)

Table 5: Serious Adverse Events^a (SAEs)

		Conditioning ^b		Follow up ^b		Total	
Duration (range), days							
Number of:		SAE	Patients	SAE	Patients	SAE	Patients
Total SAEs		44	18	32	14	76	23
Infectious SAEs		14	9	12	8	26	13
<i>Break down for infectious SAEs</i>	<i>Viral</i>	8	5	2	2	10	5
	<i>Sepsis</i>	4	3	1	1	5	4
	<i>Localised</i>	2	1	9	7	11	8
GI SAEs		6	5	10	4	16	8
<i>Break down for GI SAEs</i>	<i>Disease flare</i>	1	1	7	4	8	5
	<i>Non-flare Symptoms</i>	5	4	3	1	8	5
Hematologic SAEs		3	2	3	1	6	2
<i>Break down for haematologic SAEs</i>	<i>Anaemia</i>	2	1	3	1	5	1
	<i>Neutropenia</i>	0	0	0	0	0	0
	<i>Pancytopenia</i>	1	1	0	0	1	1
Fever SAEs		3	3	1	1	4	4
Renal SAEs		5	2	0	0	5	2
Respiratory SAEs		3	3	1	1	4	4
Other		10	6	5	4	15	9

a Serious Adverse Event is an adverse event, adverse reaction or unexpected adverse reaction, respectively, that does not necessarily have a causal relationship to the treatment, and that at any dose:

- Results in death
- Is life threatening
- Requires hospitalisation or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or
- Consists of a congenital anomaly/birth defect is otherwise medically significant

b Conditioning phase covers 100 days from start of conditioning. Follow up phase covers period from end of conditioning phase to one-year assessment

